

FORM PTO-1390		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			4121-128
			U.S. APPLICATION NO. (if known, see 37 CFR 1.5) <b>09/890745</b>
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED	
PCT/DE00/00365	4 February 2000	5 February 1999	
TITLE OF INVENTION			
FLOW-THROUGH DEVICE AND ITS USE FOR BINDING POLYMERS TO MEMBRANE SURFACES			
APPLICANT(S) FOR DO/EO/US			
Stefan Matysiak			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</li> <li>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).*(<b>Unsigned</b>)</li> <li>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol> <p><b>Items 11. to 16. below concern other document(s) or information included:</b></p> <ol style="list-style-type: none"> <li>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li>14. <input type="checkbox"/> A substitute specification.</li> <li>15. <input checked="" type="checkbox"/> A small entity statement.</li> <li>16. <input type="checkbox"/> Other items or information: EPO Search Report in German, Amended claims, translated, pursuant to Rule 66 PCT</li> </ol>			

NOTE: This application is being filed with an unsigned Oath or Declaration under the provisions of 37 CFR § 1.53 in order that applicant may secure a filing date of August 2, 2001. Upon receipt of a "Notice to File Missing Parts - Filing Date Granted," a executed Declaration and Power of Attorney will be forwarded. The undersigned agent affirmatively states that she has been duly authorized and appointed to file this application on behalf of the applicant and applicant's assignee, and that the Declaration and Power of Attorney to be filed hereafter will confirm the undersigned agent's authorization and appointment. Applicants are considered a small entity and assignee Deutsches Krebsforschungszentrum is also considered a small entity within the meaning of 37 CFR § 1.9.

17. <input checked="" type="checkbox"/> The following fees are submitted: <b>Basic National Fee</b> (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO .....\$860.00  International preliminary examination fee paid to USPTO (37 CFR 1.482) .....\$0.00 No International preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .....\$0.00  Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....\$1000.00  International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) .....\$0.00  <div style="text-align: right;"><b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b></div>				CALCULATIONS <div style="font-size: 2em; font-weight: bold; margin: 10px 0;">09/890745</div>		PTO USE ONLY	
				\$ 860.00			
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$			
Claims	Number Filed	Number Extra	Rate				
Total Claims	13-20 =	0	X \$18.00	\$			
Independent Claims	1-3 =	0	X \$80.00	\$			
Multiple dependent claim(s) (if applicable)			+ \$270.00	\$			
<b>TOTAL OF ABOVE CALCULATIONS =</b>				860.00			
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				\$ 430.00			
<b>SUBTOTAL =</b>				\$ 430.00			
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 Months from the earliest claimed priority date (37 CFR 1.492(f)).				\$			
<b>TOTAL NATIONAL FEE =</b>				\$ 430.00			
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$			
<b>TOTAL FEE ENCLOSED =</b>				\$ 430.00			
				Amount to be:	\$		
				refunded			
				Charged	\$		

a. ☒ A check in the amount of \$430.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-3284. A duplicate copy of this sheet is enclosed.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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PATENT TRADEMARK OFFICE  
09/890745  
4121-128

PATENT APPLICATION

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**In re Application of:** Stefan Matysiak

**Application No.:** New U.S. National Stage Application of  
PCT International Application No.  
PCT/DE00/00365

**International Filing Date:** 4 February 2000

**Priority Date Claimed:** 5 February 1999 (German Appl. No. 199 04  
784.7)

**U.S. National Phase Filing Date:** Date of mailing identified below

**Title:** **FLOW-THROUGH DEVICE AND ITS USE  
FOR BINDING POLYMERS TO  
MEMBRANE SURFACES**

**EXPRESS MAIL CERTIFICATE**

I hereby certify that I am mailing the attached documents to the  
Commissioner for Patents on the date specified, in an envelope  
addressed to the Commissioner for Patents, Box Patent Application,  
Washington, DC 20231, and Express Mailed under the provisions of  
37 CFR 1.10

Blake Crouch

Name of Person Mailing This Document

*Blake Crouch*  
Signature

August 2, 2001

Date

EL 831358293US

Express Mail Label Number

**PRELIMINARY AMENDMENT**

Commissioner for Patents  
BOX PATENT APPLICATION  
Washington, D.C. 20231

Sir:

0950745 "ST406860"

Prior to examination of the above-identified new national phase patent application, please amend the application, as follows:

**In the Claims**

Please amend claims 1-12 to read as follows:

- 1) A flow-through device having at least a three-part design and a sequence of parts I, II, and III, comprising:
  - part I which comprises a plurality of holes arranged side by side and each hole sealed on its bottom side by a sealing ring;
  - part II, positioned below part I and communicatively connected thereto, which comprises a coarsely porous membrane; a plurality of holes arranged in a 1<sup>st</sup> recess side by side and each sealed by a sealing ring; and a suction passage in a 2<sup>nd</sup> recess for applying a vacuum; and
  - part III, positioned below part II and communicatively connected thereto, which comprises a plurality of holes arranged in a 1<sup>st</sup> recess side by side and each sealed by a sealing ring; and at least one connection for applying a vacuum for moving fluids through the three-part design device.
- 2) The flow-through device according to claim 1, further comprising a synthesis membrane capable of binding polymers, wherein the synthesis membrane is disposed between part I and part II.
- 3) The flow-through device according to claim 2, wherein the synthesis membrane is functionalized.
- 4) The flow-through device according to claim 2, wherein the synthesis membrane is made of a material selected from the group consisting of nylon, polyamide, cellulose, polypropylene, PTFE, polyolefin, polyethylene or polystyrene, polyvinylidene fluoride, glass fiber, PVC, polymethylpentene, and polynorbornene copolymer.

- 5) The flow-through device according to claim 3, wherein the synthesis membrane comprises a functional group selected from the group consisting of hydroxyl, amino, amide, phosphate, alkyl, halogen, carboxyl, carbonyl, thio, aryl groups, ethene groups and ethyne groups.
- 6) The flow-through device according to claim 1, wherein part III includes two connections for applying a vacuum.
- 7) The flow-through device according to claim 1, wherein the coarsely porous membrane in part II is a material selected from the group consisting of polyethylene, polypropylene, PTFE and Delrin.
- 8) The flow-through device according to claim 1, wherein the flow-through device is composed of an inert material.
- 9) The flow-through device according to claim 8, wherein the inert material is selected from the group consisting of Delrin, PTFE and ceramic material
- 10) Use of the flow-through device according to claim 1 for binding polymers to membrane surfaces.
- 11) Use of the flow-through device according to claim 10 for membrane-bound molecule libraries.
- 12) Use according to claim 11, wherein the molecule libraries comprise a member selected from the group consisting of DNA, RNA, amino acids, peptides, proteins, and nucleic acid analogs

Please add claim 13.

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**REMARKS**

A marked-up version of amended paragraph in the specification and amended claims 1-13 are included herewith in Appendix A.

It is requested that the examination and prosecution of this application proceed on the basis of the English translation of the PCT International application included herewith and these amended claims 1-13.

Respectfully submitted,



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## APPENDIX A

### In the Claims

Please amend claims 1-12 to read as follows:

- 1) A flow-through device having at least a three-part design and a sequence of parts I, II, and III, [wherein] comprising:
 

part I which comprises [contains] a plurality of [drilled] holes arranged side by side and each hole sealed on its [their] bottom side by a sealing ring [each,];

part II, positioned below part I and communicatively connected thereto, which comprises [contains] a coarsely porous membrane; a plurality of [drilled] holes arranged in a 1<sup>st</sup> recess side by side and each sealed by a sealing ring [each,]; and a suction passage [or channel] in a 2<sup>nd</sup> recess for applying a vacuum[,]; and

part III, positioned below part II and communicatively connected thereto, which comprises [includes] a plurality of [drilled] holes arranged in a 1<sup>st</sup> recess side by side and each sealed by a sealing ring [each,]; and at least one connection for applying a vacuum for moving fluids through the three-part design device.
- 2) The flow-through device according to claim 1, further comprising [wherein] a synthesis membrane capable of binding polymers wherein the synthesis membrane is disposed between part I and part II.
- 3) The flow-through device according to claim 2, wherein the synthesis membrane is functionalized.
- 4) The flow-through device according to claim 2 [or 3], wherein the synthesis membrane is made of a material selected from the group consisting of nylon,



polyamide, cellulose, polypropylene, PTFE, polyolefin, polyethylene [or] and polystyrene, polyvinylidene fluoride, glass fiber, PVC, polymethylpentene, and [or] polynorbornene copolymer.

- 5) The flow-through device according to claim 3 [or 4], wherein the synthesis membrane comprises a functional group selected from the group consisting of hydroxyl, amino, amide, phosphate, alkyl, halogen, carboxyl, carbonyl, thio, aryl groups, ethene groups [or] and ethyne groups.
- 6) The flow-through device according to claim 1 [any one of the preceding claims], wherein part III includes two connections for applying a vacuum.
- 7) The flow-through device according to claim 1 [any one of the preceding claims], wherein the coarsely porous membrane in part II is a material selected from the group consisting of [consists] of polyethylene, polypropylene, PTFE [or] and Delrin.
- 8) The flow-through device according to claim 1 [any one of the preceding claims], wherein the flow-through device is composed of an inert material.
- 9) The flow-through device according to claim 8, wherein the inert material is selected from the group consisting of Delrin, PTFE [or] and ceramic material
- 10) Use of the flow-through device according to claim 1 [any one of the preceding claims] for binding polymers to membrane surfaces.
- 11) Use of the flow-through device according to claim 10[, wherein the design of] for membrane-bound molecule libraries.[ is concerned].
- 12) Use according to claim [10 or] 11, wherein the [biopolymers or] molecule libraries comprise a member selected from the group consisting of DNA, RNA,

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**Flow-Through Device and Its Use for Binding Polymers to  
Membrane Surfaces**

The present invention relates to a flow-through device and its use for binding polymers to membrane surfaces, the position of said polymers on said surfaces being determined via x, y parameters. In particular, this flow-through device is used with a method of synthesizing membrane-bonded molecule libraries.

The solid-phase synthesis of oligomers or smaller organic compounds to swellable or non-swellaable carrier materials usually takes place on resins of relatively inert polymers (e.g. highly cross-linked polystyrene) which are produced as small monodisperse globules or powders having a defined number of functional groups on their surface. Having concluded the synthesis or corresponding deprotecting procedures taking place in separate reaction chambers, these products can be collected in suitable vessels. The original grid, i.e. the array during the synthesis, is lost in this case or has to be restored by expensive and time-consuming measures, e.g. by repipetting. It is thus almost impossible to synthesize whole molecule libraries by means of this method.

There is also the synthesis of spatially addressable combinatorial libraries, i.e. molecule libraries in which the information on the sequence or the chemical steps carried out are determined via the x, y array. In particular, the parallel synthesis of molecule arrays according to the SPOT method (Frank, R., Tetrahedron 48, pp. 9217-9232, 1992) on porous membranes has to be emphasized here. Its main drawbacks are the considerable time required and the only insufficiently developed automation degree.

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It is therefore the object of the present invention to provide a device by which an automated method for binding polymers can be carried out, in particular for synthesizing molecule libraries.

The synthesis of molecule libraries on membrane surfaces is automated using a flow-through device according to the invention.

Flow-through devices are only used for filtration purposes thus far and are obtainable from the company of Schleicher & Schüll, 37582 Dassel, for example.

A flow-through device is advantageously used in the form of a synthesis block according to figure 1. The individual parts of the synthesis block are shown in figures 2a - 2e. This synthesis block distinguishes itself in particular by the use of an inert material, preferably Delrin, PTFE or ceramic materials.

The synthesis block shown in figure 1 distinguishes itself by a three-part structure, a synthesis membrane being arranged between part I and part II.

Part I contains drilled holes which are arranged side by side and have an internal diameter of about 3 mm, and the diameters of the drilled holes may, of course, have any size suitable for the respective application. Each drilled hole is sealed on the bottom side by a sealing ring, e.g. made of PTFE/silicone. The number of drilled holes is at least 96, preferably 384 or more.

Part II has preferably the following design: a coarsely porous membrane or plate, e.g. made of PE, PP, PTFE or Delrin, which has a thickness of several millimeters, preferably 2-15 mm, most preferably 4-10 mm, is disposed below the x, y holes of part I as a support for the preferably functionalized synthesis membrane which is located between parts I and II. The pore size of the coarsely porous membrane is preferably 100 to 250 µm,

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preferably 120 to 200  $\mu\text{m}$ . In a first recess of part II, a corresponding number of drilled holes is found which as in part I is also at least 96, preferably 384 or more, and which holes are also sealed with a sealing ring each, e.g. made of PTFE/silicone. In a second recess of part II a suction channel for applying a vacuum was provided for better flushing and suction. Due to this design of part II, in particular to the coarsely porous membrane as a support for the synthesis membrane, an applied vacuum is also extended to those regions which are located beyond the sealing rings. The collection and spreading of undesired reagents is reduced in this way. Based on this modification it is also possible to use several reactor types (96-well, 384-well) with a single base (parts II and III).

Part III contains a device in the form of at least one suction member to be able to apply a vacuum to the apparatus. Like parts I and II, part III has drilled holes arranged side by side, which preferably correspond to those of parts I and II as regards number and size. A preferred modification is the presence of a second vacuum channel in part III, which shall prevent reagents from collecting on the outer edge of the membrane. Both vacuum regions (vacuum chamber below the membrane, vacuum channel for the marginal regions) are separated by seals, preferably made of silicone, and can be operated with different partial vacuums.

The apparatus is assembled by superposition of the parts, safety means, e.g. in the form of clasps, being also present for locking.

The synthesis membrane located between parts I and II is suitable for binding polymers and has a pore size of 0.1 to 1.3  $\mu\text{m}$ , preferably 0.2 to 1.0  $\mu\text{m}$ . Such a membrane may consist of all materials common in this field and should preferably carry on its surface functional groups such as hydroxyl, amino, amide, phosphate, alkyl, halogen, carboxyl, carbonyl, thio, aryl groups, ethene groups (e.g. vinyl,

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vinylloxy, vinylloxy carbonyl and the corresponding pure thio or mixed thio analogs) or ethyne groups. Nylon, polyamide, cellulose, polypropylene, PTFE, polyolefin, polyethylene or polystyrene, polyvinylidene fluoride, glass fiber, PVC, polymethyl pentene, polynorbornene copolymers (e.g. topas, Hoechst company) are suitable as basic materials of the membrane. The membrane used is most preferably a membrane made of surface-oxidized (hydrophilized) polypropylene which was derivatized correspondingly.

In a preferred way, the membrane has compartments which form automatically once because the membrane is clamped into the synthesis block and constrictions form by the sealing rings on the bottom side of part I of the synthesis block, which effect a lateral boundary of the round reaction surfaces. The reagents to be applied in series, in particular the activated monomers in the production of a molecule library, are then added in accurate doses to avoid lateral contaminations. The volume supplied should be such that the corresponding spots do not flow into one another. All parallel chemical steps, e.g. application of the wash solutions, are made in excess, i.e. the chemicals distribute over all reaction chambers, but are sucked off downwardly through the support by an applied volume. Another vacuum is preferably applied laterally to suck off excess reagent (cf. figure 2e).

On the other hand, it is possible to apply regions formed by welding on the membrane beforehand, which correspond exactly to the holes on parts I and II of the synthesis block and which have to be fitted thereto. As a result, an array identical with the common synthesis formats results which has relatively small membrane surfaces delimited against one another. This functions by inserting the membrane in the synthesis block. Individual separate reaction chambers form by forcing the sealing rings onto the membrane and applying an increased pressure and/or temperature or by sealing using an inert thermoplastic material (e.g. low-melting polypropylene granulate) below the seals. Reagents which

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shall be applied in series or parallel can then also be applied in excess without a lateral contamination resulting because the membrane compartments are sealed against one another. A "fried egg" structure results whose confined units have the advantage as regards avoiding contaminations and spreading reactants over the entire membrane.

Reagents are pipetted via conventional synthesizers (e.g. spot synthesizer of the company of Abimed Analysentechnik, Langenfeld, Germany) into the wells of part I of the synthesis block on the membrane surface where they are covalently bonded by a chemical reaction. Excess reagents or wash solutions are sucked off using a vacuum. As a result, the cycle time becomes much shorter, the binding of the polymers to the membrane surface and thus the establishment of a molecule library is faster many times over. In addition to the minimized synthesis time, the still present membrane structure enables the complete membrane to be subjected to a screening method after the concluded synthesis (e.g. hybridization of a radioactively labeled DNA probe due to specific base pairing). This takes place without the oligomers being transferred to other vessels as done in the prior art or having to be anchored on other carriers. This ensures that e.g. the entire molecule library can be checked for specific interactions under exactly the same conditions. Polymers are preferably understood to mean DNA, RNA, amino acids, peptides, proteins, nucleic acid analogs (e.g. PNA, LNA).

The invention is described in more detail by means of the figures, in which:

Figure 1 shows a synthesis block

Figure 2 shows the design of the synthesis block

- a: synthesis block, part I, top view
- b: synthesis block, part I, bottom side
- c: synthesis block, part I, bottom side, drilled hole enlarged

- d: synthesis block, part II, top view
- e: synthesis block, part III, top view

Figure 3 shows

- a: membrane hybridized with  $d(T)_{16}$   $^{33}P\gamma ATP$
- b: membrane additionally hybridized with  $d(C)_{16}$   $^{33}P\gamma ATP$
- c: control.

The invention is described in more detail by means of an example:

Example:

Membrane: hydrophilized polypropylene, pore size 0.2  $\mu m$ , reacted with trisamine Jeffamine 500 (bifunctional aminopolyethylene glycol) after carbonyldiimidazole activation  
concentration: 0.12  $\mu mol/cm^2$

Pretreatment: membrane with a mixture of 2 ml NMP, 33  $\mu l$  diisopropylcarbodiimide (DIC), 62.0 mg Fmoc- $\beta$ -Ala-OH, 27.0 mg HOAt welded in and incubated at 37°C for 3 h.

Reaction cycle:

- capping in 20 ml DMF + 600  $\mu l$  acetic anhydride; 30"
- capping in 20 ml DMF + 600  $\mu l$  acetic anhydride; 2"
- 2 x washing in DMF, 2' and 5'
- 2 x washing in ethanol, 2' and 5'
- 2 x washing in DMF, 2' and 5'
- deprotecting in 20 % piperidine in DMF
- 2 x washing in DMF, 2' and 5'
- 2 x washing in ethanol, 2' and 5'
- 2 x washing in DMF, 2' and 5'



- staining in 20 ml DMF + 600 µl BPB  
parent solution, 10 mg/ml
- destaining in ethanol
- drying.

Of all amino acids to be spotted 0.3 molar parent solutions in NMP were prepared and stored on a molar sieve at 4°C.

Prior to spotting, the respective parent solution was heated and mixed in a ratio of 1:1:1 with 0.3 molar HOAt parent solution and 0.4 molar DIC parent solution. Of this solution 0.2 µl were applied per spot onto the membrane. This was repeated three times, the reaction time after each application was 20 to 40 minutes each.

A screen or raster of 8x12 was spotted in the microtitration plate format.

After each spotting cycle, the following reaction cycle adapted to the apparatus according to figure 1 was run.

- capping in 20 ml DMF + 600 µl acetic anhydride (+ 600 µl pyridine); 200 µl per spot; reaction time 10', sucking off
- 2 x washing in DMF; 200 µl per spot; sucking off permanently
- 2 x washing in ethanol; sucking off permanently
- 2 x washing in DMF; sucking off permanently
- deprotecting in 20 % piperidine in DMF; reaction time 10', sucking off
- 2 x washing in DMF; 200 µl per spot; sucking off permanently
- 2 x washing in ethanol; sucking off permanently
- sucking to dryness for at least 45'.

The following linker molecules are applied:

- Fmoc Rink Handle (in array 1, lines 1-4) or Fmoc-β-Ala-OH (in arrays 2 and 3, lines 5-8 and 9-12)
- Fmoc-Lys(dansyl)-OH.

Thereafter, 10 cycles were spotted with Fmoc-A(aeg)-OH (lines 1, 5, 9); Fmoc-C(aeg)-OH (lines 2, 6, 10); Fmoc-G(aeg)-OH (lines 3, 7, 11) or Fmoc-T(aeg)-OH (lines 4, 8, 12).

The membrane was divided into the 3 arrays, and arrays 1 and 3 were frozen.

Final treatment for array 2:

Array 2 was incubated for 10 min. at room temperature in 9.5 ml TFA, 500 µl triisobutylsilane (separation of the lateral protecting groups), washed 2 x in DMF and 1 x in ethanol and dried.

Thereafter, array 2 was incubated in hybridization solution (d(T)<sub>16</sub> <sup>33</sup>PyATP) for 30' at room temperature and washed. The membrane was then exposed overnight on a phosphorimager screen. The picture of figure 3a showed.

The membrane was then washed and additionally incubated in hybridization solution (d(C)<sub>16</sub> <sup>33</sup>PyATP for 30' at room temperature, washed and exposed on a phosphorimager screen for 4 days. The signals are marked as shown in figure 3b.

The efficiency of the first linkage can be determined by means of the fluorescence intensity of the first building block attached by condensation. In this case, Fmoc-Lys(dansyl)-OH was used as the first building block. In case this fluorescence label is only used during the synthesis at certain points, the condensation yield can be determined via the relative intensity. The picture of figure 3c shows at 353 nm (ordinary U.V. lamp). It follows therefrom that the distribution of the spots took place at certain points and no cross-contamination occurs.

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**Claims**

- 1) A flow-through device having at least a three-part design (Parts I - III), wherein
  - part I contains drilled holes arranged side by side and sealed on their bottom side by a sealing ring each,
  - part II contains a coarsely porous membrane; drilled holes arranged in a 1<sup>st</sup> recess side by side and sealed by a sealing ring each; and a suction passage or channel in a 2<sup>nd</sup> recess for applying a vacuum,
  - part III includes drilled holes arranged in a 1<sup>st</sup> recess side by side and sealed by a sealing ring each, and at least one connection for applying a vacuum.
- 2) The flow-through device according to claim 1, wherein a synthesis membrane capable of binding polymers is disposed between part I and part II.
- 3) The flow-through device according to claim 2, wherein the synthesis membrane is functionalized.
- 4) The flow-through device according to claim 2 or 3, wherein the synthesis membrane is made of nylon, polyamide, cellulose, polypropylene, PTFE, polyolefin, polyethylene or polystyrene, polyvinylidene fluoride, glass fiber, PVC, polymethylpentene, or polynorbornene copolymer.
- 5) The flow-through device according to claim 3 or 4, wherein the synthesis membrane comprises hydroxyl, amino, amide, phosphate, alkyl, halogen, carboxyl, carbonyl, thio, aryl groups, ethene groups (e.g. vinyl, vinyloxy, vinyloxycarbonyl groups and the corresponding

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pure thio analogs or mixed thio analogs) or ethyne groups.

- 6) The flow-through device according to any one of the preceding claims, wherein part III includes two connections for applying a vacuum.
- 7) The flow-through device according to any one of the preceding claims, wherein the coarsely porous membrane in part II consists of polyethylene, polypropylene, PTFE or Delrin.
- 8) The flow-through device according to any one of the preceding claims, wherein the flow-through device is composed of an inert material.
- 9) The flow-through device according to claim 8, wherein the inert material is Delrin, PTFE or ceramic material.
- 10) Use of the flow-through device according to any one of the preceding claims for binding polymers to membrane surfaces.
- 11) Use according to claim 10, wherein the design of membrane-bound molecule libraries is concerned.
- 12) Use according to claim 10 or 11, wherein the polymers or molecule libraries comprise DNA, RNA, amino acids, peptides, proteins, nucleic acid analogs.

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**Amended Claims**

- 1) A flow-through device having at least a three-part design and a sequence of parts I, II, III, wherein
  - part I contains drilled holes arranged side by side and sealed on their bottom side by a sealing ring each,
  - part II contains a coarsely porous membrane; drilled holes arranged in a 1<sup>st</sup> recess side by side and sealed by a sealing ring each; and a suction passage or channel in a 2<sup>nd</sup> recess for applying a vacuum,
  - part III includes drilled holes arranged in a 1<sup>st</sup> recess side by side and sealed by a sealing ring each, and at least one connection for applying a vacuum.
- 2) The flow-through device according to claim 1, wherein a synthesis membrane capable of binding polymers is disposed between part I and part II.
- 3) The flow-through device according to claim 2, wherein the synthesis membrane is functionalized.
- 4) The flow-through device according to claim 2 or 3, wherein the synthesis membrane is made of nylon, polyamide, cellulose, polypropylene, PTFE, polyolefin, polyethylene or polystyrene, polyvinylidene fluoride, glass fiber, PVC, polymethylpentene, or polynorbornene copolymer.
- 5) The flow-through device according to claim 3 or 4, wherein the synthesis membrane comprises hydroxyl, amino, amide, phosphate, alkyl, halogen, carboxyl, carbonyl, thio, aryl groups, ethene groups or ethyne groups.
- 6) The flow-through device according to any one of the

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preceding claims, wherein part III includes two connections for applying a vacuum.

- 7) The flow-through device according to any one of the preceding claims, wherein the coarsely porous membrane in part II consists of polyethylene, polypropylene, PTFE or Delrin.
- 8) The flow-through device according to any one of the preceding claims, wherein the flow-through device is composed of an inert material.
- 9) The flow-through device according to claim 8, wherein the inert material is Delrin, PTFE or ceramic material.
- 10) Use of the flow-through device according to any one of the preceding claims for binding polymers to membrane surfaces.
- 11) Use according to claim 10, wherein the design of membrane-bound molecule libraries is concerned.
- 12) Use according to claim 10 or 11, wherein the biopolymers or molecule libraries comprise DNA, RNA, amino acids, peptides, proteins, nucleic acid analogs.

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**Synthesis block**

Part I: 16 x 24 (384) continuous or straight drilled holes, approximately 3 mm in diameter

Part II: 16 x 24 (384) straight drilled holes, approximately 3 to 0.5 mm in diameter

Part III: vacuum chamber

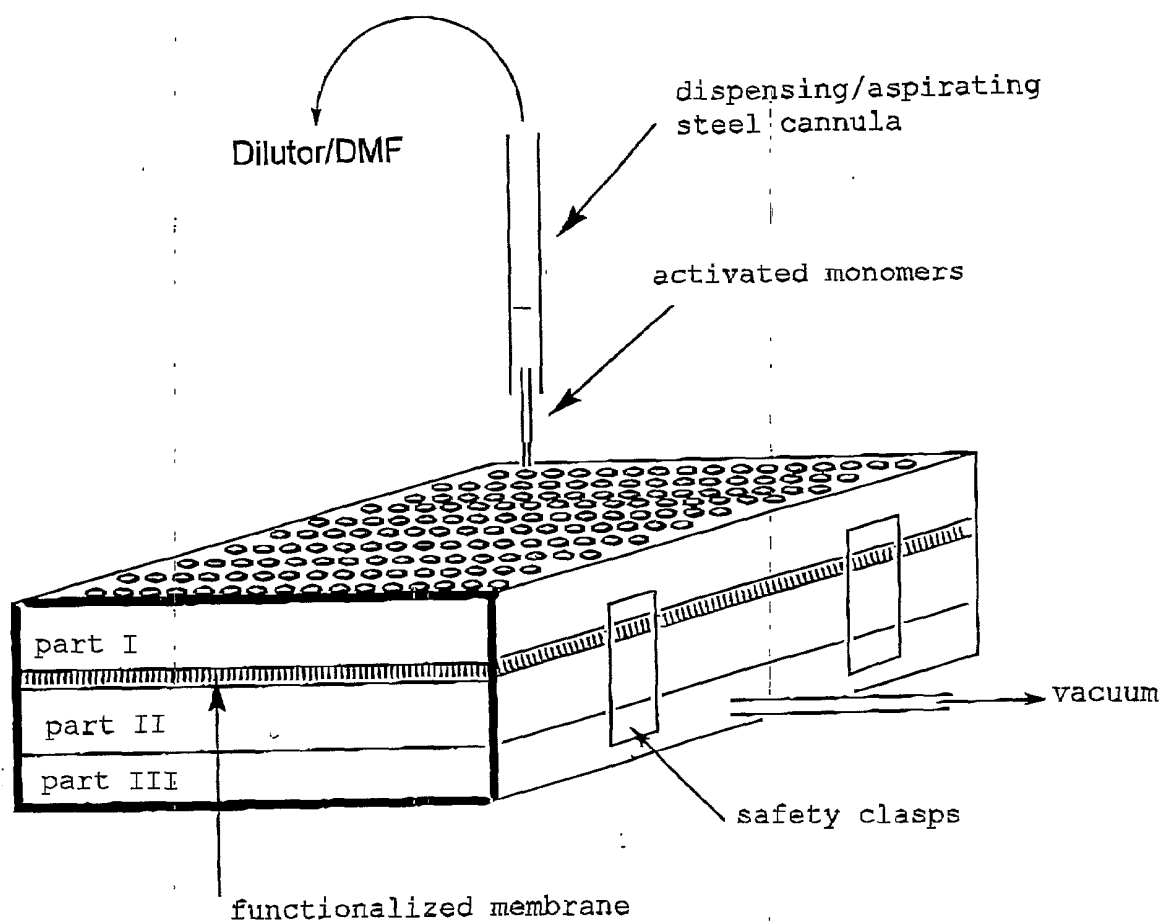
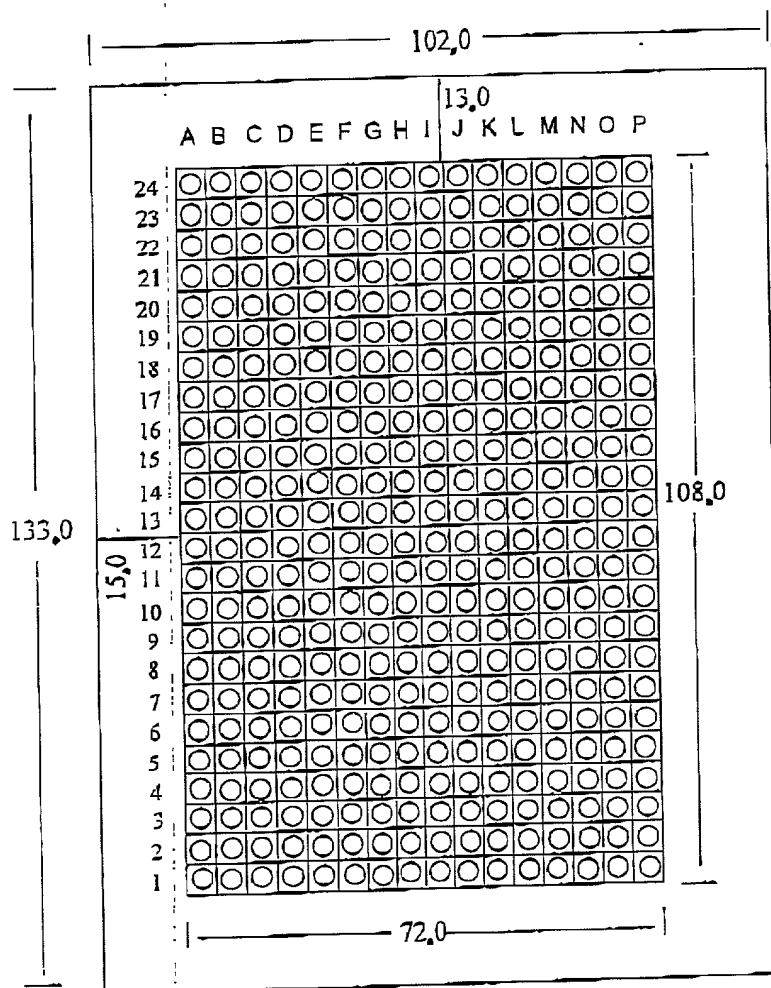


Fig. 1

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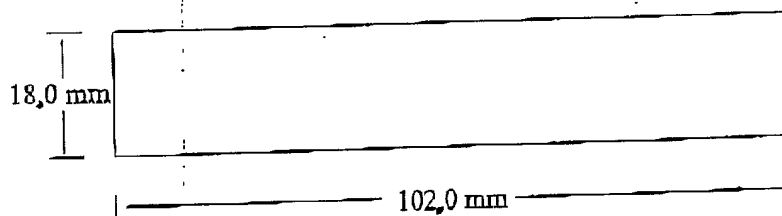
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Synthesis block (16 x 24 = 384), part I, top view



top view:

- drilled hole
- top side
- 3 mm in diameter



side view:

All indications made in mm; with 3.0 mm outside diameter there is a lateral distance of altogether  $72 - 48 = 24$  mm or  $108 - 72 = 36$  mm, i.e.  $24/16 = 36/24 = 1.50$  mm distance from the outer edge of hole 1 to the outer edge of hole 2; the distance is 0.7-0.5 mm on the bottom side with 3.8-4.0 mm sunk diameter drilled

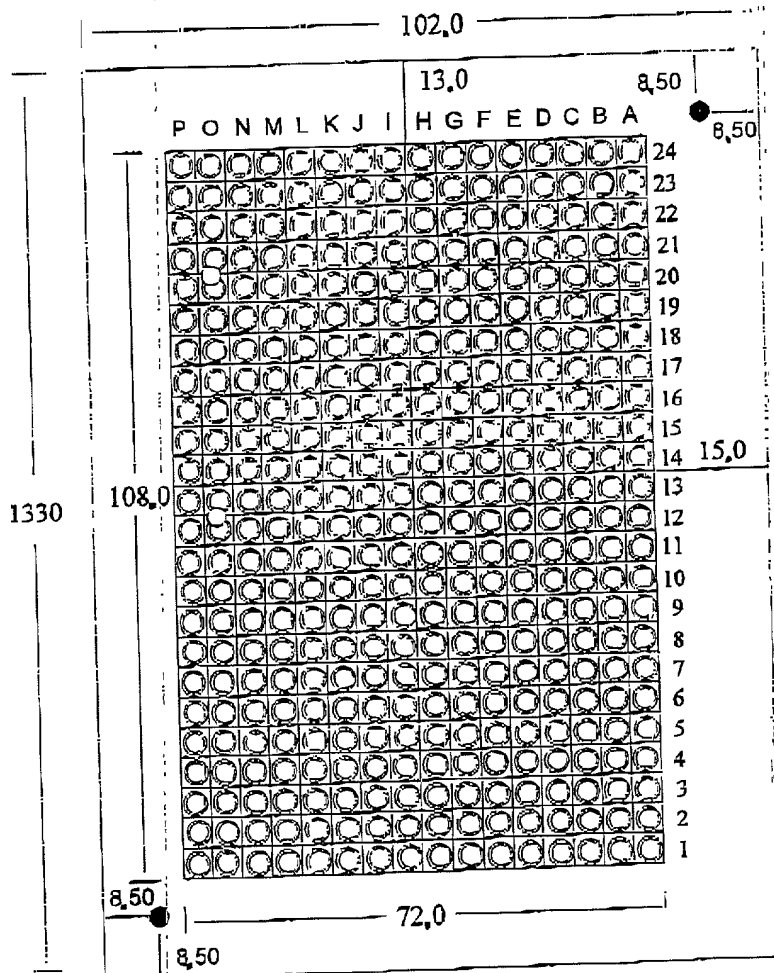
Fig. 2a



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Synthesis block (16 x 24 = 384), part I, bottom side



Bottom side:

- drilled hole for fixing pin  
diameter 3.50 mm  
hole depth 6.00 mm
- drilled hole bottom side  
sunk hole 1.0 mm  
3.00 mm inside diameter  
4.00 mm outside diameter
- sealing ring of PTFE/silicone  
inside diameter 3.00 mm  
outside diameter 4.00 mm

All indications made in mm.

Fig. 2b

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Synthesis block (16 x 24 = 384), part I, bottom side  
Drilled hole enlarged

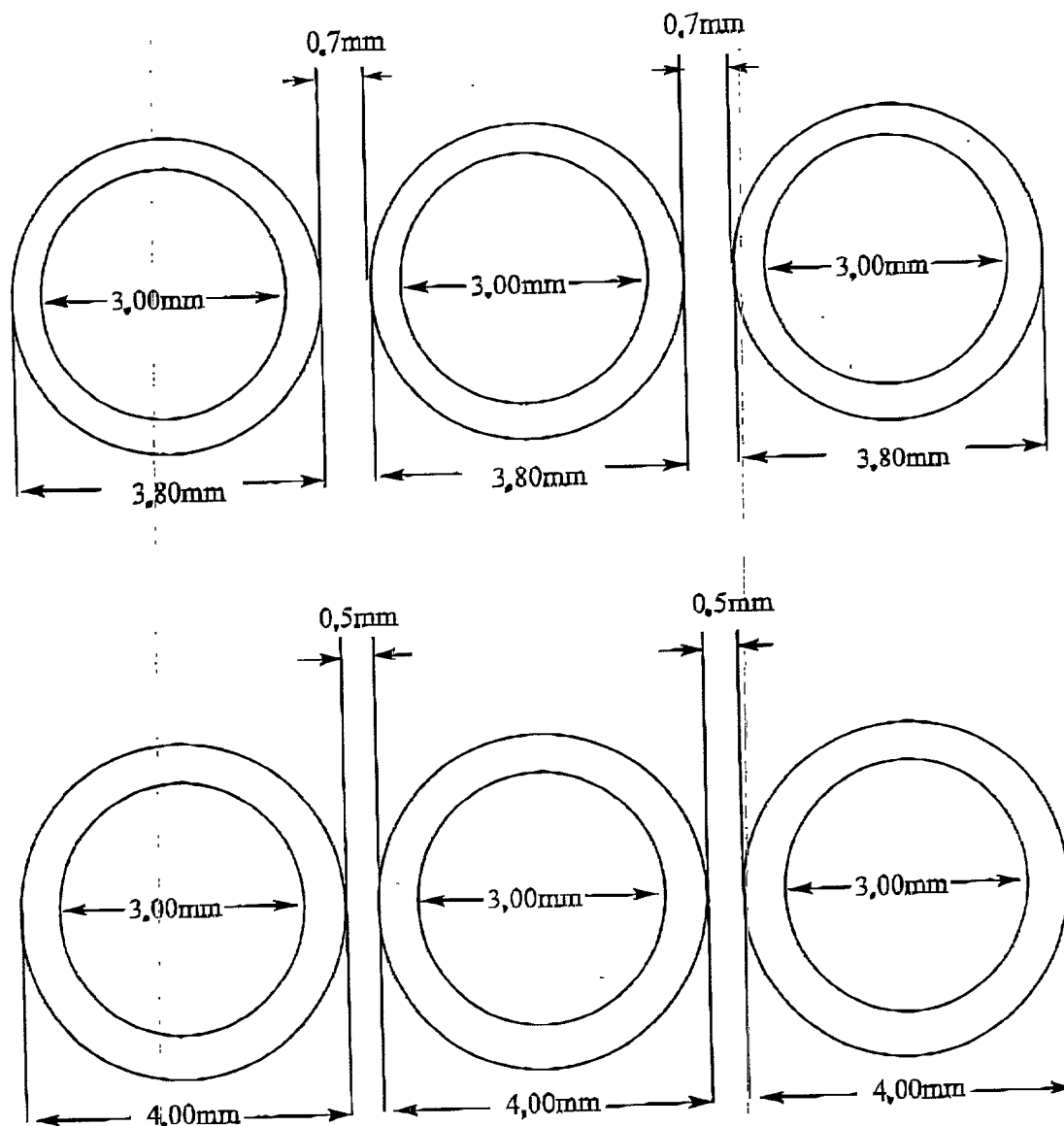


Fig. 2c

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Flow-through synthesis block, prototype II, part II, top view

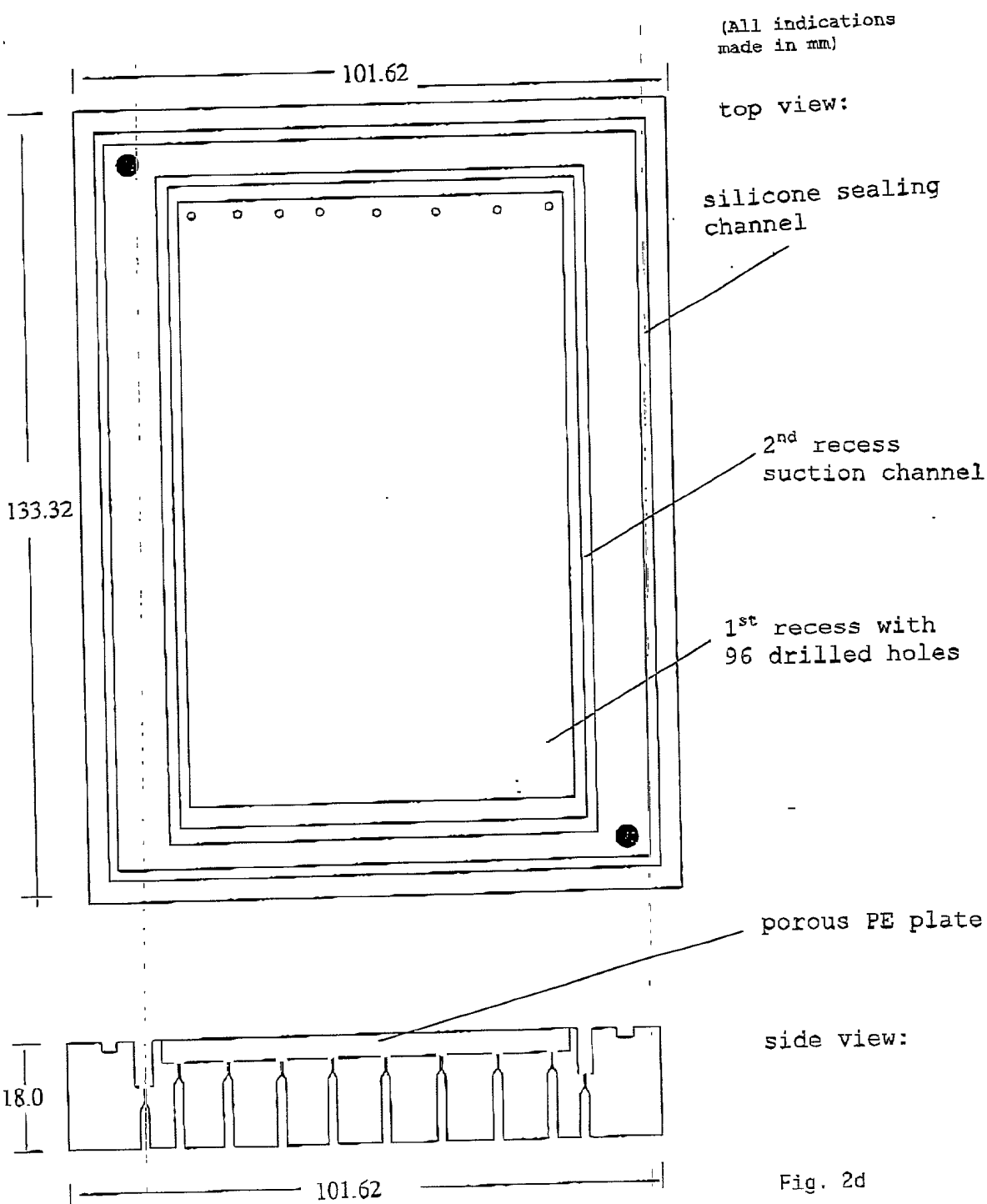


Fig. 2d

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## Flow-through synthesis block, prototype II, part III, top view

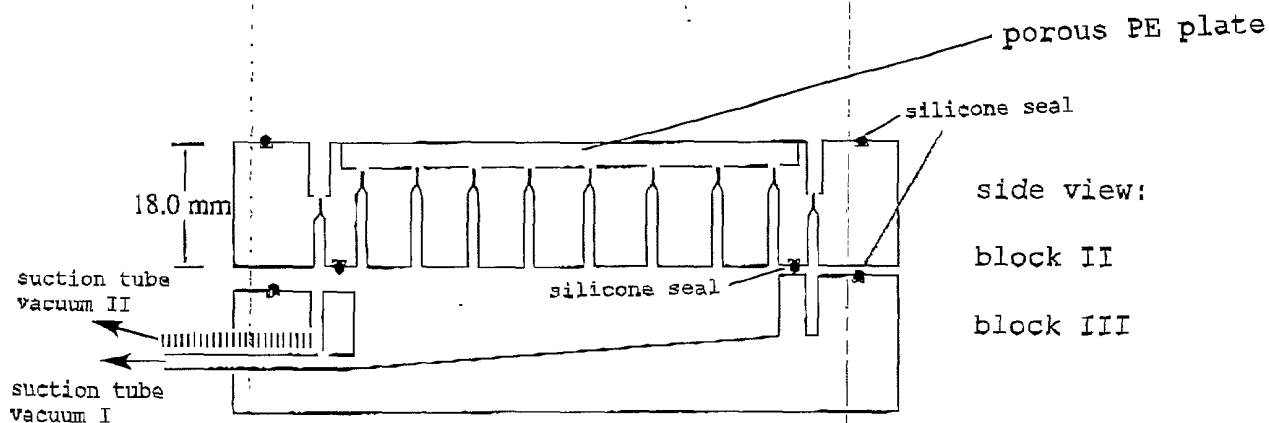
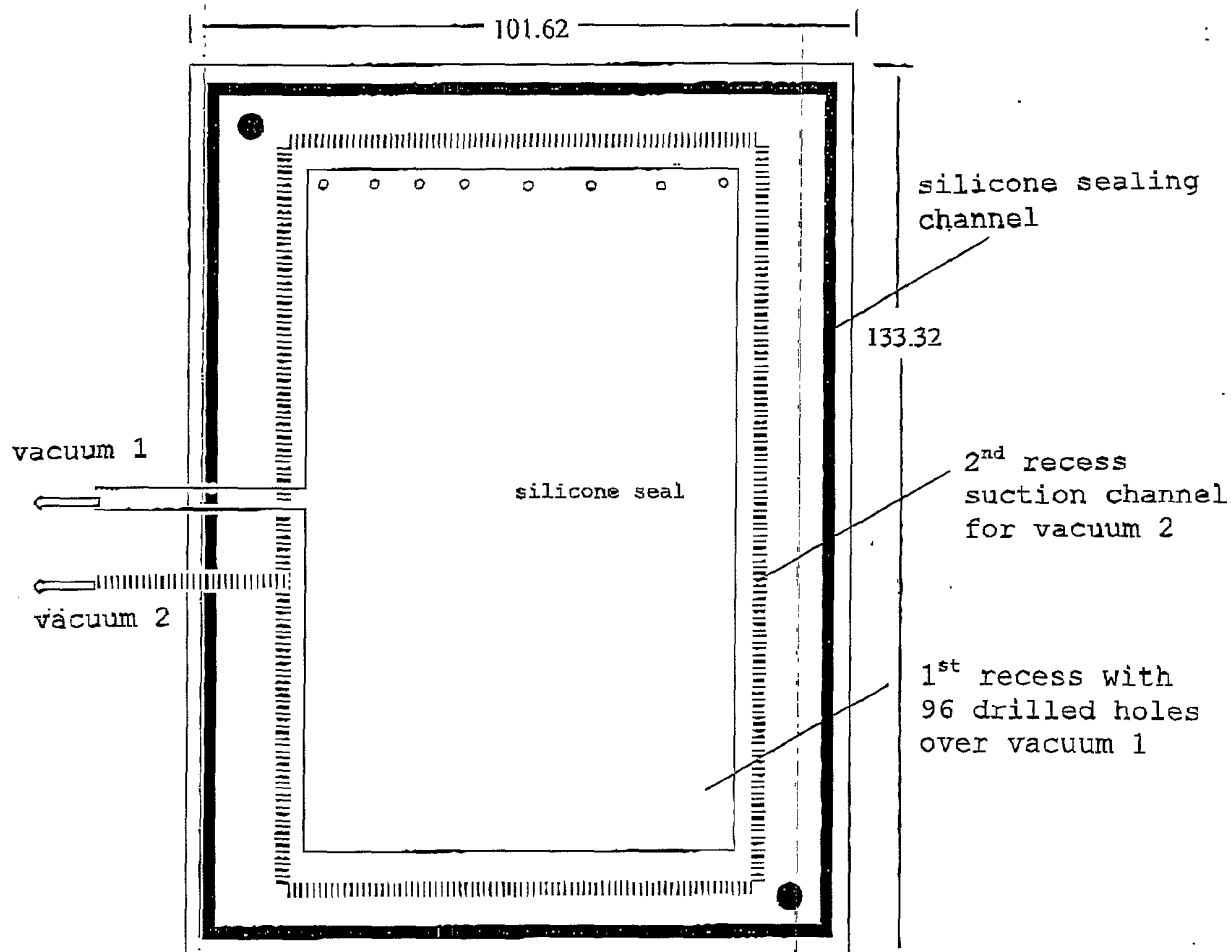
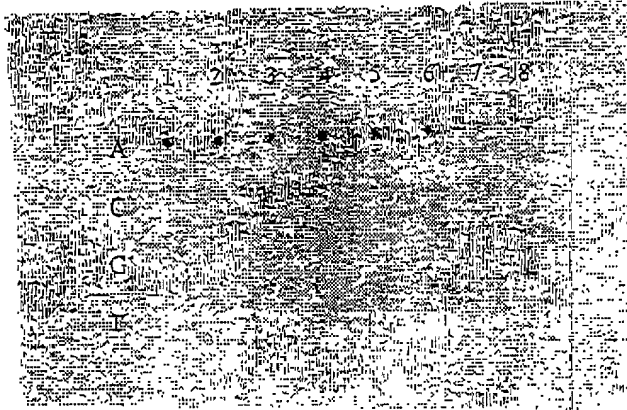


Fig. 2e

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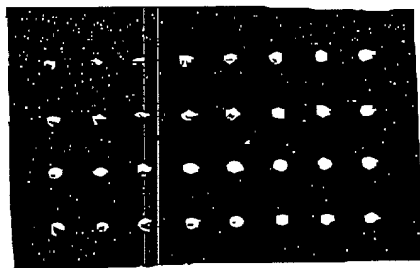


(a)

	1	2	3	4	5	6	7
A	•	•	•	•	•	•	•
C							
G	•	•	•	•	•	•	•
T							

(b)

column 8 was by mistake not detected!



(c)

Fig. 3

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## PATENT APPLICATION

DECLARATION AND POWER OF ATTORNEY  
FOR PATENT APPLICATION

ATTORNEY DOCKET NO. 4121-128

As a below named inventor, I hereby declare that:

My residence/post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter, which is claimed and for which a patent is sought on the invention entitled:

FLOW-THROUGH DEVICE AND ITS USE FOR BINDING POLYMERS TO MEMBRANE SURFACES

the specification of which is attached hereto unless the following box is checked:

(X) was filed August 2, 2001 as US Application Serial No. 9/890,745 or PCT International Application

Number \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understood the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose all information which is material to patentability as defined in 37 CFR 1.56.

## Foreign Application(s) and/or Claim of Foreign Priority

I hereby claim foreign priority benefits under Title 35, United States Code Section 119(a-d) or 365(b) of any foreign application(s) for patent or inventor(s) certificate, or 365(e) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor(s) certificate having a filing date before that of the application on which priority is claimed:

COUNTRY	APPLICATION NUMBER	DATE FILED	PRIORITY CLAIMED UNDER 35 U.S.C. 119
Germany	199 04 784.7	5 February 1999	YES: <u>X</u> NO: _____
PCT	PCT/DE00/00365	4 February 2000	YES: <u>X</u> NO: _____

## Provisional Application

I hereby claim the benefit under Title 35, United States Code Section 119(e) of any United States provisional application(s) listed below:

## U.S. Priority Claim

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NUMBER	FILING DATE	STATUS(patented/pending/abandoned)

## POWER OF ATTORNEY:

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) listed below to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Steven J. Hultquist, Reg. No. 28,021Marianne Fuierer, Reg. No. 39,983

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## Send Correspondence to:

Steven J. Hultquist  
Intellectual Property/Technology Law  
P.O. Box 14329  
Research Triangle Park, NC 27709

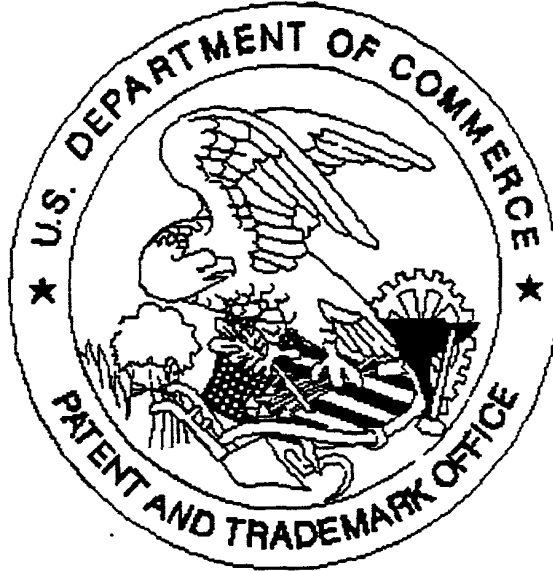
## Direct Telephone Calls To:

Steven J. Hultquist  
(919) 419-9350

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of Inventor: Stefan MatysinkCitizenship: GermanResidence: In der Neckarhelle 11, D-69118 Heidelberg, GermanyPost Office Address: SameStefan Matysink  
Inventor's Signature11.10. 2001  
Date

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